Vol. XXII. *April, 1912.* *No. 5*

BIOLOGICAL BULLETIN

THE EFFECTS OF SOME AMIDO-ACIDS ON THE DEVELOPMENT OF THE EGGS OF ARBACIA AND OF CHÆTOPTERUS.

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In 1909, Mathews published a short account of some experiments which he made to ascertain the effects of various amido-acids on the development of the eggs of *Arbacia*. The results of these experiments have considerable theoretical interest, since they seem to show that the course of embryonic development can be determined, to a greater or a less extent, by these products of protein digestion.

While I was working in the Marine Biological Laboratory at Woods Hole, Mass., in the summer of 1909, Dr. Mathews kindly furnished me with a number of amido-acids in order that I might repeat and extend his experiments and make a detailed study of the different types of larvæ that might be obtained. As it seemed worth while to determine whether amido-acids can alter the course of development in various kinds of eggs or whether they have a specific action on the eggs of *Arbacia*, the experiments were carried beyond the limits originally intended and were made with the eggs of an annelid, *Chætopterus pergamentaceus*, as well as with the eggs of the sea-urchin, *Arbacia punctulata*.

In addition to cystin, leucin and tyrosin, the three amido-acids which Mathews used in his experiments, both kinds of eggs were subjected to the action of glutamic acid, aspartic acid, asparagine, glycocoll and alanin. In each series of experiments eggs from two or more females were thoroughly mixed and then artificially fertilized in sea-water. As soon as the polar bodies had been

extruded, approximately equal portions of the eggs were transferred into finger bowls which contained 100 c.c. of the solution to be tested. As a control by which to judge of the effects of the solutions, one portion of the eggs was allowed to develop in 100 c.c. of normal sea-water. The various experiments were made in a similar manner and the eggs were kept under like conditions of light and of temperature during their development in order that the results of the experiments might not be affected by environmental conditions other than those that were being studied.

A. Experiments with the Eggs of *Arbacia punctulata*.

As the breeding season of *Arbacia* is near its close the latter part of July, only a small number of eggs suitable for experimental purposes could be obtained. All of the eggs used were presumably in a normal physiological condition, as at least 90 per cent. of those in the control cultures developed in a normal manner and became plutei.

In each series of experiments observations were made at frequent intervals on the living embryos. These observations were later supplemented by a microscopic study of various lots of material that had been fixed in corrosive sublimate and stained with Heidenhain's iron-hæmatoxylin or with Delafield's hæmatoxylin followed by eosin.

Cystin ($C_6H_{12}O_4N_2S_2$).—As this substance is very insoluble in cold sea-water, the solution used in the first experiment that was made was prepared in the following way: A quantity of the pure crystalline salt was placed in a flask of sea-water heated to 40° C. The mixture remained at this temperature for one half hour and was then sealed and set aside. After three days the solution was filtered, to remove the undissolved cystin, and used within a few hours.

A lot of *Arbacia* eggs was fertilized at 11.45 A.M. on the morning of July 14, 1909, and a portion of them was placed in the saturated solution of cystin at 12.15 P.M. These eggs were found to be segmenting in a normal manner when division of the eggs in the control culture took place at 12.50 P.M., and for some hours the eggs of both cultures seemed to be developing at about

the same rate. If the cystin had any effect on the segmentation it was too slight to be detected either in the living eggs or in preserved material.

On the morning of July 15, both cultures contained many living embryos; those of the control were well-developed gastrulæ that were swimming at the surface of the water in a normal manner; those in the cystin solution were decidedly smaller than the control larvæ, and most of them were swimming at the bottom of the dish. Thirty hours after the experiment was started all of the larvæ in the cystin solution were dead, although the larvæ in the control culture were still in good condition. Preserved material showed that the development of the eggs that had been subjected to the action of the cystin solution took place in a perfectly normal manner, although it was somewhat slower than that of the eggs in the control lot.

Mathews found that cystin produced a decided acceleration in the development of the eggs of *Arbacia*, which was apparent from the fourth division on. The solution that he used was made as follows: "One hundred centimeters of sea-water were shaken for a moment with about a centigram of crystalline cystin and the mixture poured into a finger bowl with the undissolved cystin. The eggs, fertilized something less than an hour before, were then added and the eggs lay during development among the crystals of cystin at the bottom of the dish." As a solution made in this way is undoubtedly much weaker than that employed in my first experiment, it seemed probable that the opposing results obtained by Mathews and myself might be due to the difference in the strength of the solutions to which the eggs were subjected. The experiment was therefore repeated with a different lot of eggs, the solution of cystin that was used being prepared in the manner described by Mathews.

In this experiment, also, the development of the eggs appeared to progress at about the same rate in both the cystin culture and in the control. Some of the eggs in the cystin solution seemed to segment much more rapidly than others, and a very few of them developed at a faster rate than the major portion of the eggs in the control culture. A careful comparison between the two cultures, made at intervals of about one half hour during the

entire day, failed to show any marked acceleration in the development of the great majority of the eggs in the cystin solution. Twenty hours after the experiment began swimming larvæ were found at the surface in both cultures, so in this instance the development of the blastulæ was not retarded by the cystin. The solution was ultimately harmful, however, as all of the larvæ in the cystin culture died within thirty-six hours, while those of the control developed into plutei that lived for several days. No unusual types of larvæ were noted among the living forms, and none were found in microscopic preparations of the older embryos.

The *Arbacia* eggs with which Mathews experimented were undoubtedly in a very different physiological condition from those that I used, for Mathews states that in the control lots for his experiments "hardly a pluteus was to be found and these few were generally abnormal." In both of my control cultures the great majority of the eggs formed normal plutei that lived for some days. With such a great difference in the lots of eggs experimented upon it is not surprising that the results do not agree, since the reaction of eggs to any external stimulus depends, to a considerable extent, upon the particular physiological conditions existing in the eggs at the time that the stimulus is applied.

Leucin ($C_6H_{13}NO_2$).—By the use of a weak solution of "impure leucin" Mathews changed the course of development of the eggs of *Arbacia* so that many of the embryos were totally unlike *Arbacia* larvæ. "In many, evagination of the entoderm instead of invagination, took place. A few developed a ciliated band in the shape of the star-fish bipinnaria. . . . Another form was perfectly spherical with a single ciliated band about the middle. It looked in its external form like a small trochophore." Unfortunately, it was not possible to obtain any of the impure leucin with which Mathews produced these remarkable forms of *Arbacia* larvæ, and the leucin with which I experimented was presumably pure.

Solutions of various strengths (2, 1, $\frac{1}{4}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) were used on batches of eggs that were fertilized at 11.30 A.M. on the morning of July 16, 1909. The eggs in all of the cultures began segmenting at the same time as those in the control lot,

but the stronger solutions very soon proved toxic and greatly retarded development. None of the eggs in the 2 per cent. solution of leucin had developed beyond the 2-cell stage at the time that the great majority of the eggs in all of the other solutions, as well as in the control, were in the 8-cell stage. A solution of this strength, however, does not kill the eggs quickly, as twenty hours after the experiment began this culture contained a few ciliated larvæ that were much smaller, and less active, than those of the control lot. Within twenty-four hours all of the larvæ in the 2 per cent. solution of leucin were dead.

A microscopic examination was made of a large number of eggs taken from the 2 per cent. solution of leucin at different stages in their development. Many of the young eggs were abnormal in that there was an irregular distribution of the chromosomes to the poles of the segmentation-spindle or a very unequal division of the blastomeres. Such abnormal eggs evidently died before reaching the blastula stage, as nearly all of the older embryos that were examined were normal although somewhat smaller than those of the control culture. A few abnormal blastulæ were found among the older larvæ, but as these larvæ showed only such irregularities of form as may be found in individuals of almost every control culture of *Arbacia* larvæ developing in a small amount of sea-water under laboratory conditions, they could not be considered as due to the specific action of the leucin in changing the course of development.

The eggs in the 1 per cent. solution of leucin began to show the injurious effects of the solution after the first hour, and from this time on their development, although normal, lagged behind that of the control: the weaker solutions had apparently no effects on the early segmentation. The blastulæ in the control culture began moving about fifteen minutes sooner than the larvæ in the other cultures, so evidently all of the leucin solutions retarded development somewhat after the first two or three hours. Plutei that seemed perfectly normal, and that lived for several days, developed in all of the weaker solutions. An examination of a considerable number of these embryos, preserved at various stages in their development, failed to show any larvæ that were in any way comparable to the unusual types that Mathews obtained with impure leucin.

A second experiment was made with leucin on July 24, 1909. In this instance a solution of the strength of ½ per cent. was employed, since stronger and weaker solutions do not alter the course of development. From the beginning of the experiment the segmentation of these eggs lagged behind that of the eggs in the control lot, and the retardation in development was fully as great as that produced by the 1 per cent. solution of leucin in the former series of experiments. Later the development of these eggs progressed at a more normal rate, and after seven hours the embryos appeared nearly as well developed, and fully as vigorous, as those in the control. The next morning larvæ were swimming at the surface in both cultures, but those in the leucin solution soon dropped to the bottom of the dish and began to disintegrate. Microscopic preparations showed that the very great majority of these larvæ were normal in every respect.

Mathews states that in the summer of 1908, when his experiments were made, the sea-urchin eggs showed in many instances the remarkable peculiarity, recorded by Mathews and Whitcher ('03), that "a large number of eggs while living for several days not forming plutei, or but a small per cent. of irregular plutei." The experiments which Mathews made to test the action of amido-acids on the development of the eggs of *Arbacia* were made therefore, wholly or in great part, on eggs that were in a peculiar physiological condition when experimented upon: whether they could be considered as normal is doubtful. The unusual types of larvæ that Mathews obtained by treating eggs with a weak solution of impure leucin were probably due to abnormal or unusual conditions existing in the eggs at the time of their fertilization, and not to the specific actions of leucin in changing the course of development. The effects of leucin on eggs of *Arbacia* that are in a normal physiological condition when fertilized depends chiefly upon the strength of the solution used: a strong solution retards development and causes the early death of the embryos; a weak solution permits of normal development at first and is toxic only after many hours.

Tyrosin ($C_9H_{11}NO_3$).—This substance is not very soluble in cold sea-water, and in order to obtain a solution of sufficient strength one gram of tyrosin crystals was put into 100 c.c. of sea-water and

the mixture brought to the boiling point. The solution was then cooled to laboratory temperature, filtered, and used at once.

The early development of the eggs used in this experiment was normal, although slightly delayed. After twenty hours ciliated larvæ were present in great number in the solution, but they were moving feebly and beginning to show degenerative changes. Prepared material showed that tyrosin had retarded the development of the eggs but produced no abnormalities. These results agree with those obtained by Mathews in a similar experiment.

Glutamic Acid ($C_5H_9NO_4$).—Various solutions of this substance (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) were used on the eggs of *Arbacia*, and all of them proved to be injurious from the beginning of the experiment. The eggs placed in the stronger solutions (1 and ½ per cent.) were killed at once. A few of the eggs subjected to the action of the $\frac{1}{10}$ per cent. solution began to segment in a normal manner, but none of them developed beyond the early stages of segmentation. The eggs in the $\frac{1}{30}$ per cent. solution continued to live for some time, but their development was very greatly retarded and stopped entirely when the gastrula stage was reached. Preparations of these eggs showed that the effects of the glutamic acid was to check development, not to produce unusual types of larvæ.

Aspartic Acid ($C_4H_7NO_4$).—This substance has a more deleterious action on the eggs of arbacia than has glutamic acid. All of the eggs placed in a 1 per cent. solution and in a ½ per cent. solution were killed at once; those subjected to the action of a $\frac{1}{10}$ per cent. solution did not develop beyond the 2-cell stage. A solution of the strength of $\frac{1}{30}$ per cent. allowed a considerable number of the eggs to develop to the blastula stage, but segmentation was very irregular and much slower than that of the eggs in the control culture.

Preparations of various lots of eggs that had been treated with aspartic acid solutions showed abnormal conditions not found in any of the *Arbacia* eggs subjected to the action of other amido-acids. Most of the eggs that had been subjected to the action of a $\frac{1}{10}$ per cent. solution of aspartic acid for four hours before fixation were found to be still unsegmented, and many of them had been entered by several spermatozoa. Only one sperm-

nucleus had fused with the egg-nucleus, however, and the segmentation-spindle that was formed usually appeared normal, although in many cases it occupied a very eccentric position close to the periphery of the egg. All of the accessory spermatozoa at this time were in the form of a small, rounded nuclei that were scattered throughout the cytoplasm.

The $\frac{1}{30}$ per cent. solution of aspartic acid had a different action on different eggs, depending, doubtless, upon the condition of the eggs when they were placed in the solution. Five hours after the experiment was begun about one fourth of the eggs were still unsegmented; some of the eggs were just beginning to segment; while others were in later stages of segmentation, and the cleavage planes were coming in very irregularly in many cases. A very few eggs had reached the blastula stage at this time, but they were not as well developed as the eggs in the control lot. After twenty-two hours the number of eggs that had reached the blastula stage was found to be consideraly increased. Development had been checked by this time, however, and the greater number of larvæ appeared as more or less irregular masses of cells that were beginning to disintegrate.

Preparations of this material showed many cases of polyspermy. Some of the unsegmented eggs contained a large multipolar segmentation-spindle formed, evidently, by the fusion of several sperm-nuclei with the egg-nucleus: other eggs contained a segmentation-spindle of the normal size with the chromosomes very unequally distributed to the spindle poles. The condition of these eggs greatly resembled that which O. and R. Hertwig ('87) found could be induced in fertilized echinoderm eggs by subjecting them to the action of various chemical substances which prevented their normal development.

Asparagine ($C_4H_{10}N_2O_4$).—This amide of aspartic acid proved to be far less injurious to the eggs of *Arbacia* than did the latter substance, when used in solutions of the same strength (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.). The great majority of the eggs in all of the cultures began to segment at the normal time and in a normal manner. After two hours the eggs in the 1 per cent. solution showed evidence of retarded development, but the eggs in all of the other solutions developed at a normal rate for some hours.

Twenty-four hours after the experiment began, ciliated larvæ were present in great numbers in all of the solutions, but they all died many hours before the death of the larvæ in the control culture.

Glycocoll ($C_2H_5NO_2$).—This substance, which is the simplest of the amido-acids, was much less harmful to the eggs of *Arbacia* than were any of the other amido-acids used in these experiments. During the first twenty-four hours the development of the eggs did not appear to be affected in any way by the solutions used (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.), but during the second day the embryos began to show degenerative changes, and all of them died about fifty hours after the experiment began. Sections of these eggs fixed at various stages of development merely confirmed the observations on the living forms, as no unusual types of larvæ were found.

Alanin ($C_3H_7NO_4$).—This amido-acid dissolves readily in cold sea-water, and it was used in solutions of the following strengths: 2, 1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent. The stronger solutions (2, 1 and $\frac{1}{2}$ per cent.) retarded development from the beginning: the weaker solutions had no apparent effects on the segmentation of the eggs. After twenty-four hours each of the solutions contained a large number of swimming larvæ, and only those in the 2 per cent. solution showed any evidence of retarded development. The embryos in all of the cultures died some hours before the death of the control larvæ; so weak solutions of alanin cannot be considered as favorable media in which to rear the eggs of *Arbacia*. Preserved material showed no abnormalities worthy of note at any stage of development.

All of the amido-acids used in this series of experiments with the eggs of *Arbacia* proved to be toxic, the injurious effects of any substance depending very largely upon the strength of the solution used. In no case was the course of development altered in a definite direction, except in the very young eggs and in these the abnormalities produced were of the types commonly found when fertilized eggs of the sea-urchin are treated with various chemical solutions.

B. EXPERIMENTS WITH THE EGGS OF *Chætopterus pergamentaceus.*

As the eggs of *Chætopterus* could be obtained in considerable numbers at Woods Hole in the summer of 1909, experiments were made to study the influence of amido-acids on the early development of this annelid, in the hope that some definite alterations in development might be produced comparable to those obtained by Loeb ('01) and by Lillie ('02) when eggs of *Chætopterus* were treated with potassium salts. Material intended for microscopic study was preserved in Boveri's picric-acetic solution and stained with hæmatoxylin.

Cystin.—On the morning of August 6, 1909, a lot of *Chætopterus* eggs was placed in 100 c.c. of a saturated solution of cystin as soon as the polar bodies had been extruded. The early development of these eggs was slightly accelerated, and swimming larvæ were found in this culture nearly one half hour before any movement could be detected in the control larvæ. The next day the cystin solution was swarming with well-developed trochophores, but they all died about fifty hours after the experiment began. No abnormal embryos were noted at any stages of development and none were found in preserved material.

The experiment was repeated several days later with eggs from another female. The results obtained were practically the same as in the first experiment, since there was more rapid development during the segmentation period. The solution proved to be toxic after thirty hours, however, killing the embryos without producing any alterations in structure.

Leucin.—In one series of experiments this substance was used on the eggs of *Chætopterus* in solutions of the following strengths: $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{80}$ per cent. None of these solutions had any marked effects on the early segmentation of the eggs, but they evidently caused a slight acceleration in development during a later period as the larvæ in all of the solutions began moving some thirty minutes before there was any movement of the control larvæ. Twenty hours after the experiments were started all of the cultures were carefully examined. The majority of the eggs that had been treated with the ½ per cent. solution had stopped their development in the blastula stage, and were lying at the bottom of the dish apparently dead; a very few larvæ were swimming

at the surface of the solution, but they had evidently reached their maximum development and would soon die. The $\frac{1}{10}$ per cent. solution contained a considerable number of swimming larvæ, but these larvæ were not in good condition and plainly showed the injurious effects of the leucin. A large number of ciliated embryos were found in the $\frac{1}{30}$ per cent. solution, and they appeared somewhat further advanced in development than those in the control culture. Degenerative changes appeared in these larvæ in about twenty-four hours, however, and all of them were dead within thirty hours. No unusual types of larvæ were found in preparations of these eggs fixed at various stages in their development.

As it seemed possible that the solutions of leucin employed in the experiments described above might have been too weak to produce any alteration in the development of the eggs, a second experiment was made in which a batch of eggs was subjected to the action of a 1 per cent. solution of leucin. These eggs segmented at the normal time, but two hours later their development was found to be lagging behind that of the eggs in the control culture. After four hours the retardation in development was very marked, and in some instances two or more eggs had fused together. Loeb and Lillie have noted that the fusion of several embryos into giant forms is a phenomenon of frequent occurrence when eggs of *Chætopterus* are treated with potassium salts. In twenty hours all of the larvæ were dead, and so disintegrated that it was impossible to preserve any material fit for study. Sections of eggs fixed in earlier stages of development failed to show any abnormalities except the occasional fusion of two or more embryos.

Tyrosin.—This substance was used on the eggs of *Chætopterus* in a saturated solution which is less than $\frac{1}{10}$ per cent. Only a very few of the eggs had segmented when the first division occurred in the control eggs. After four hours the tyrosin culture showed all stages in development from the unsegmented egg through to late segmentation, the most advanced eggs being apparently at the same stage of development as the eggs of the control. All of the embryos in the tyrosin solution died within twenty-four hours after the experiment was started. Preserved

material showed that tyrosin acts on the eggs of *Chætopterus* as it does on the eggs of *Arbacia*, causing a marked retardation in development but producing no specific abnormalities.

Glutamic Acid.—Solutions of various strengths (1, $\frac{1}{2}$ and $\frac{1}{10}$ per cent.) were used, the eggs being placed in the solutions about three quarters of an hour after their fertilization. All of the eggs in the two stronger solutions were evidently killed at once as none of them made any attempts to divide. Some of the eggs in the $\frac{1}{10}$ per cent. solution began to elongate after the solution had acted upon them for one hour, and later many of these eggs took on an irregular shape as if attempting to divide into several cells at the same time. None of these eggs had segmented after five hours, however, so they were all returned to normal sea-water in the hope that they would then be able to continue their development. There was no segmentation of any of the eggs, although they appeared to live for some hours.

Sections of preserved material showed that the segmentation-spindle had formed in many eggs in an apparently normal manner, but that development had been stopped at this point.

Aspartic Acid.—Eggs of *Chætopterus* fertilized at 10.55 A.M. on August 8, 1909, were placed in solutions of aspartic acid (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) at 11.25 A.M. The eggs in the control culture were segmenting at 11.55 A.M., but no evidence of cleavage could be detected in any of the eggs in the aspartic acid solutions until 1.30 P.M., when a few of the eggs in the $\frac{1}{30}$ per cent. solution began to elongate as if about to divide. A number of these elongated eggs were isolated and carefully watched for some time, but in no case did any division occur. Sections of preserved material showed that some eggs contained a normal segmentation-spindle, while others had a multipolar spindle that occupied an eccentric position close to the periphery. The stronger solutions of aspartic acid killed the eggs before the formation of the segmentation-spindle.

Asparagine.—Solutions of this substance of the same strengths as those used in the experiments with aspartic acid were tested. Normal cleavage began in the eggs of all of the cultures at the same time as in those of the control lot. Observations made at frequent intervals during the next four hours showed that seg-

mentation was progressing in a normal manner and at about the same rate in all of the solutions.

Five hours after the eggs had been fertilized a few larvæ in the $\frac{1}{10}$ per cent. solution were moving slowly: at this time there was no movement of any of the embryos in the other cultures or in the control lot. A weak solution of asparagine, therefore, slightly accelerates the development of the eggs of *Chætopterus*, if it be that an earlier movement of the embryos is indicative of a more advanced stage of development. At the end of the sixth hour the effects of the various solutions were very marked: the embryos in the $\frac{1}{10}$ per cent. solution were moving more actively than those in the control, and they seemed slightly better developed; the larvæ in the other solutions were moving slowly and their development lagged considerably behind that of the control larvæ. After eight hours the larvæ in the 1 per cent. solution were all at the bottom of the dish and evidently dying; no abnormal types of larvæ could be detected among the living forms, and none were found in preserved material that was examined later. The embryos in the other solutions were swimming at the surface after ten hours, but none of them lived more than twenty-four hours.

Glycocoll.—In the strengths of solutions used (1, $\frac{1}{2}$ and $\frac{1}{10}$ per cent.), this substance did not appear to have any effects whatever on the eggs during the first twelve hours. On the second day the larvæ began dying, and all of them had been killed by the end of the third day.

Alanin.—Batches of *Chætopterus* eggs that had been artificially fertilized at 10.30 A.M. on the morning of August 8, 1909, were put into various solutions of alanin (1, $\frac{1}{2}$, $\frac{1}{10}$ and $\frac{1}{30}$ per cent.) at 11 o'clock. The eggs in all of the cultures, including the control, began segmenting at the same time, and all of them developed at about the same rate during the next two hours. At 3.30 P.M. a number of swimming larvæ were found in the $\frac{1}{10}$ and in the $\frac{1}{30}$ per cent. solutions, but at this time there was no movement of the larvæ in any of the other cultures. At 4.30 P.M. ciliated larvæ were present in great numbers in all of the solutions; but the larvæ in the 1 per cent. solution could move but slowly, and soon all of them sank to the bottom of the dish and disintegrated.

At 9 A.M. on the morning of August 9, the larvæ in the ½ per cent. solution were dying, and a number of giant embryos had been formed by the fusion of two or more of the larvæ: the embryos in the $\frac{1}{10}$ per cent. and in the $\frac{1}{30}$ per cent. solutions were apparently normal and were moving vigorously. All of the larvæ were dead on the morning of August 10, although the trochophores in the control culture were still very active at this time. Preserved material showed no abnormalities worthy of note.

As weak solutions of alanin did not seem to affect the early development of the eggs adversely a second series of experiments was made in which batches of *Chætopterus* eggs were treated with 4 per cent. and with 2 per cent. solutions of alanin as soon a they had extruded their polar bodies.

None of the eggs in the 4 per cent. solution segmented, and sections of preserved material showed that the eggs had been killed before the formation of the segmentation-spindle. When cleavage began in the eggs of the control lot at 11 A.M. a very few of the eggs in the 2 per cent. solution were dividing in an apparently normal manner; in the great majority of the eggs segmentation was very greatly delayed. After four hours only a few eggs had reached the 4-cell stage, and in these eggs the cleavage planes had come in very irregularly. An hour later development had stopped entirely and the eggs were fusing into large, irregularly shaped masses. At this time the eggs were transferred into normal sea-water in the hope that segmentation might be resumed, but although the eggs seemed to live for some hours, none of them developed beyond the 4-cell stage.

In microscopic preparations of eggs that had been in the 2 per cent. solution of alanin for two hours before fixation only a very few normal 2-cell stages were found, and the great majority of the eggs contained a multipolar spindle with the chromosomes very irregularly distributed along the spindle fibres. Material fixed after the solution had acted for five hours showed that only the first cleavage in any of the eggs was normal and that in most eggs development had stopped at this point. Where further division had occurred the blastomeres were very irregular in size and shape, and although hundreds of eggs were examined no stage later than an 8-cell stage could be found.

When multipolar spindles formed in the eggs as a result of their treatment with a 2 per cent. solution of alanin the eggs, apparently, were never able to divide, although there seemed to be a long period during which active and resting stages alternated with each other. In the resting stages the eggs contained either one large, oblong nucleus, or several smaller ones that were more or less irregular in outline. In the active periods one large, multipolar spindle with hundreds of chromosomes scattered about it would be formed, or several small spindles, all more or less irregular, would be scattered throughout the cell. In some of these eggs a number of accessory asters were formed, similar to those that Morgan ('96, '99) found could be produced in the eggs of *Arbacia* and of various other forms by means of salt solutions.

A 2 per cent. solution of alanin produced greater abnormalities in the eggs of *Chætopterus* than did any of the other solutions of amido-acids that were used, but as these abnormalities were of the types that can be produced in different kinds of eggs by treatment with various salts they cannot be considered as the result of any specific action on the part of the alanin.

Summary and Conclusions.

With the exception of cystin, which is a sulphur-containing compound, all of the amido-acids used in these experiments are composed of the same chemical elements, yet they differ to a marked extent in their toxic action on developing eggs. Glutamic acid and aspartic acid are by far the most injurious, even a $\frac{1}{30}$ per cent. solution of these substances killing the eggs of both *Arbacia* and of *Chætopterus* at a very early period. Glycocoll, on the other hand, permits of the development of normal plutei and trochophores, and only injures the embryos after twenty-four hours. The other amido-acids used retard development, to a greater or less extent, depending chiefly upon the strength of the solution employed.

A brief summary of the effects of the various solutions of amido-acids on the development of the eggs of *Arbacia* and of *Chætopterus* during the first twelve hours is given in the following table. Ultimately all of the solutions are toxic, even though they appear to favor development during an early period.

TABLE I.

Amido-acid.	Solution Used.	Effects on *Arbacia* Eggs.	Effects on *Chætopterus* Eggs
Cystin	Saturated	No effects on segmentation; later development retarded.	Development accelerated.
	$\frac{1}{30}$ per cent.	Development very slightly retarded.	Development slightly accelerated.
	$\frac{1}{10}$ per cent.	Development very slightly retarded.	Development slightly accelerated.
	$\frac{1}{4}$ per cent.	Development very slightly retarded.	.....
Leucin.	$\frac{1}{2}$ per cent.	Development slightly retarded.	Development accelerated at first, but stopped in blastula stage.
	1 per cent.	Development retarded after 1 hour.	Development retarded after 2 hours; embryos fused.
	2 per cent.	Development greatly retarded; a few eggs abnormal.	.....
Tyrosin.	Saturated.	Development retarded.	Development retarded.
	$\frac{1}{30}$ per cent.	Development stopped in the gastrula stage.	.....
Glutamic acid.	$\frac{1}{10}$ per cent.	Eggs killed in early segmentation.	Eggs lived for some time, but no segmentation.
	$\frac{1}{2}$ per cent.	Eggs killed at once.	Eggs killed at once.
	1 per cent.	Eggs killed at once.	Eggs killed at once.
	$\frac{1}{30}$ per cent.	Development stopped in blastula stage; many eggs abnormal.	Eggs lived for some time, but no segmentation.
Aspartic acid.	$\frac{1}{40}$ per cent.	Development stopped at 2-cell stage; many eggs abnormal.	Eggs killed at once.
	$\frac{1}{2}$ per cent.	Eggs killed at once.	Eggs killed at once.
	1 per cent.	Eggs killed at once.	Eggs killed at once.
	$\frac{1}{30}$ per cent.	No effects noted.	.....
	$\frac{1}{10}$ per cent.	No effects notes.	Development slightly accelerated.
Asparagine.	$\frac{1}{2}$ per cent.	No effects noted.	Segmentation not affected; later development retarded.
	1 per cent.	Development retarded after 2 hours.	Segmentation not affected; later development retarded.
	$\frac{1}{30}$ per cent.	No effects noted.	No effects noted.
Glycocoll.	$\frac{1}{10}$ per cent.	No effects noted.	No effects noted.
	$\frac{1}{2}$ per cent.	No effects noted.	No effects noted.
	1 per cent.	No effects noted.	No effects noted.
	$\frac{1}{30}$ per cent.	No effects noted.	Development slightly accelerated.
Alanin.	$\frac{1}{10}$ per cent.	No effects noted.	Development slightly accelerated.
	$\frac{1}{2}$ per cent.	Development somewhat retarded.	Segmentation not affected; older embryos fused.

Amido-acid.	Solution Used.	Effects on *Arbacia* Eggs.	Effects on *Chætopterus* Eggs.
	1 per cent.	Development greatly retarded.	Development retarded after 2 hours.
Alanin.	2 per cent.	Development greatly retarded.	Development retarded; many eggs abnormal.
	4 per cent.	.....	Eggs killed at once.

As shown in the above table, all of the stronger solutions of amido-acids that were used had much the same effect on both kinds of eggs experimented upon, but several of the weaker solutions had a much more pronounced action on the eggs of *Chætopterus* than on those of *Arbacia*. Weak solutions of cystin, of leucin, of asparagine and of alanin accelerate the development of the eggs of *Chætopterus* to a noticeable extent, yet none of these solutions have apparently any effect on the early development of the eggs of *Arbacia*. The eggs of *Chætopterus* cannot segment at all when placed in a $\frac{1}{30}$ per cent. solution of aspartic acid, although this solution permits the eggs of *Arbacia* to develop to the blastula stage.

The abnormalities produced in the eggs of *Arbacia* and of *Chætopterus* by various solutions of amido-acids consist chiefly of polyspermy, irregularities in the mitotic figures, variable cleavage, and a fusion of several embryos into giant forms. No embryos were found that showed either the larval characteristics of other forms or marked peculiarities of structure that might be attributed to the specific action of the solution in which they were reared.

The results obtained in these experiments indicate that solutions of amido-acids can alter the rate at which the eggs of *Arbacia* and of *Chætopterus* develop, but that they have no influence whatever in determining the character of the development, when the eggs experimented upon are in a normal physiological condition.

LITERATURE CITED.

Hertwig, O. und R.

'87 Ueber den Befruchtungs- und Teilungsvorgang des tierischen Eies unter dem Einfluss äusserer Agenzien. Jen. Zeitschr. Naturwiss., Bd. XX., 1887.

Lillie, F. R.

'02 Differentiation without Cleavage in the Egg of the Annelid, *Chætopterus pergamentaceus*. Arch. Entwicklungsmech., Bd. XIV., 1902.

Loeb, J.

'01 Experiments on Artificial Parthenogenesis in Annelids (*Chætopterus*) and the Nature of the Process of Fertilization. Amer. Jour. Physiol., Vol. IV., 1901.

Mathews, A. P.

'09 The Influence of Some Amino-Acids on the Development of Echinoderms. Biol. Bull., Vol. XVI., 1909.

Mathews, A. P. and Whitcher, B. R.

'03 The Importance of Mechanical Shock in Protoplasmic Activity. Amer. Jour. Physiol., Vol. VIII., 1903.

Morgan, T. H.

'96 The Production of Artificial Astrospheres. Arch. Entwicklungsmech., Bd. III., 1896.

'99 The Action of Salt Solutions on the Unfertilized and Fertilized Eggs of *Arbacia*, and of Other Animals. Arch. Entwicklungsmech., Bd. VIII., 1899.

A DISCUSSION OF CYCLOPS VIRIDIS JURINE.

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Cyclops viridis Jurine, or, as it was formerly called, *Cyclops brevicornis* Claus, the object of the classic researches of Haecker, is described as being represented in North America in the form of several varieties.

C. Dwight Marsh ('10), in his revision of the North American species of *Cyclops*, divides the species *viridis* into four varieties: var. *ingens* Herrick, var. *brevispinosus* Herrick, var. *parcus* Herrick, and var. *Americanus* Marsh.

Ingens includes the largest forms of the species and possibly corresponds to the European var. *gigas* Claus.

Americanus is the most abundant variety of *viridis* in American waters. It is to be met with in almost any ditch or small pond. *Parcus* is much more local in its haunts but in the localities where it is to be found it may be abundantly represented. I have never found the two varieties together. *Parcus* is, on the average, smaller than *Americanus*.

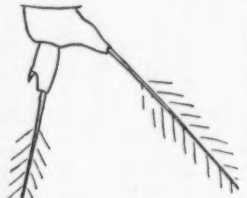
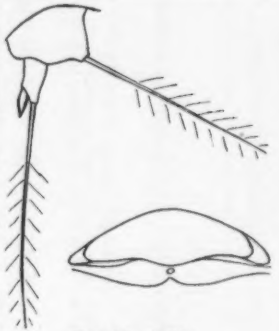
In the accompanying table I have indicated the main features distinguishing the European and the two last mentioned American varieties.

The inner margin of the furcal rami of the tail is never ciliated in the two American varieties as it is in the European form.

The rudimentary fifth thoracic appendage of the European *viridis* (see table) is described by Schmeil ('92) as having the small spine on the inner margin of the second segment either a mere cuticular protuberance or connected with that segment by a distinct joint. The latter feature is characteristic of the two American varieties (see table).

The number of spines on the terminal segments of the outer rami of the four swimming appendages in the European *viridis* and in *parcus* are two for the first pair of appendages and three

Cyclops viridis Jurine (excl. var. *gigas*, *ingens*, and *brevispinosus*).

	European Variety.	Var. *parcus* Herrick.	Var. *americanus* Marsh.
1. Fifth foot (that of the Eur. var. is drawn on a scale half of that of the other two).			
2. Receptaculum seminis (that of Eur. var. from Claus).			
3. Inner margin of furcal rami.	Ciliated.	Devoid of cilia.	Devoid of cilia.
4. Spine formula.	2, 3, 3, 3.	2, 3, 3, 3.	3, 4, 4, 4.
5. Average body-length.	2.2 mm. (Wolf)	1.4 mm.	1.5 mm.
6. Chromosome number.	12	6	10

for the next three pairs. *Americanus* has three on the first and four on the other three pairs.

E. F. Byrnes ('09) considers *parcus* and *Americanus* to be heterogenous [1] forms of the same species. This she assumes from the fact that the only fundamental difference noted between them is the armature of the swimming appendages and this appears to be variable, for occasionally one may find an adult *Cyclops* unmistakably *C. Americanus*, in which most of the swimming feet agree with *C. Americanus* in having four spines on the terminal segments of the outer rami, while others are in the condition characteristic of *C. parcus*, having but three spines on the terminal segments of the rami.

Neither Byrnes nor Marsh ('10) have remarked upon the dissimilarity in the shape of the receptaculum seminis of the two forms. Systematists working on Cyclopidæ admit that the most constant and characteristic feature for a given species is the shape of that organ. It is remarkable, therefore, that more care is not taken in figuring the seminal receptacle of described forms.

In all three varieties the receptaculum consists of a larger antero-median portion and two narrower postero-ventral portions which are carried out laterally as the sperm ducts.

Parcus possesses a receptaculum (see table) which resembles that of the European *viridis* in that the upper portion is concave anteriorly. That of *Americanus* (see table) is convex.

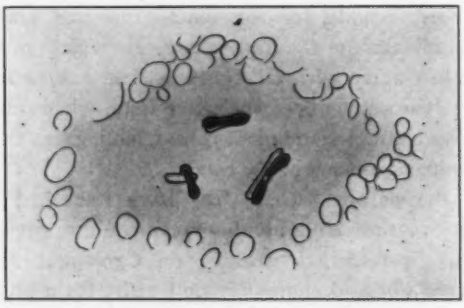
Another point which seems to leave no room for doubt as to the distinctness of the two varieties is the constant difference in their chromosome number. I have found that the somatic chromosome number in *C. Americanus* is 10 whereas in *C. parcus* it is 6.

Specimens collected in widely separated localities, as Toronto, Woods Hole, and New York, have thus far shown this difference in chromosome number to be constant for the two varieties.

A specimen taken from a pure culture of *C. parcus* showed a variation in the spines of its thoracic limbs, the spine formula being 3, 4, 4, 3 or 4 (?). A systematist would probably consider

[1] The term *heterogeny* is used here to denote the existence of two adult forms which represent successive generations, both of which are sexually mature, but morphologically unlike.

this a case of interrelationship between *C. parcus* and *C. Americanus*. That this cannot be so is to be seen from the following: I was fortunate enough to section the specimen when the chromosomes of its oviduct eggs were in the so-called "biserial arrangement" and where the count is particularly easy. The presence



Nucleus of oviduct egg of *Cyclops parcus*, showing the three pairs of chromosomes in "biserial arrangement."

of *three pairs* of chromosomes (see text figure) leaves no doubt as to the identity of the specimen.

The European *Cycl. viridis* has 12 chromosomes (Haecker, Braun).

Haecker ('97) described *Cyclops brevicornis* Claus (*viridis* Jurine) which he studied as being anywhere from 3.5–5 mm. in length. The size mentioned indicates that he was probably working with var. *gigas* Claus. It is remarkable that he gives the somatic chromosome number to be 24, although in the ovary he describes the chromosomes as bivalent, being 12 in number.

Braun ('09) also studied *Cyclops viridis* Jurine but not the variety *gigas*. He gives the somatic number of chromosomes for the typical species as 12. Unfortunately he does not mention sizes except in stating that the species varies between 1.5–5.1 mm. in length.

Schmeil ('92) gives the body length of the typical European *viridis* to be anywhere from 1.5–3.5 mm.

Wolf ('05) places the average length at 2.2 mm. Our American *parcus* on the other hand is not more than half the average size

of the European form. The specimens I have met with have never been over 1.5 mm. in length and are much more frequently between 1.2 mm. and 1.4 mm. long.

The drawings in the accompanying plate also show the disparity in size between the American and the European forms, that of the fifth foot of the European variety, from a specimen in my possession, being drawn to a scale half of that of the other drawings.

Is it not possible that we have here a case similar to that which R. R. Gates ('09) discovered in *Œnothera? Œnothera gigas*, a giant mutant of *O. Lamarckiana*, was found to possess 28 chromosomes or double the number of the parent form (14). Its cells were found to be correspondingly larger. Gates suggested that in an egg of *O. Lamarckiana* a double number of chromosomes arose from a division of the chromosomes unaccompanied by nuclear and cell division soon after fertilization and that this egg developed into the *O. gigas* form.

Either *C. parcus* or the European *C. viridis* may conceivably be a mutation one of the other if, in the one case, all of the chromosomes split into halves without subsequent nuclear division, or, in the other, go into mitosis without splitting so as to produce the number 12 for the European *viridis* or 6 for the American *parcus*. The cells of the European *viridis* containing twelve chromosomes would then be twice the size of those of *C. parcus* which has only six. The actual discrepancy in size between the two forms could thus be explained.

Note.—Since the above was sent to the printers I have secured specimens of a *Cyclops viridis* from several pools near Edgewater, N. J. They are mostly from 2–3 mm. in length although several mature individuals measure only 1.4 mm.

Not only do they resemble the typical European *viridis* Jurine in size; in the ciliated inner margin of the furcal rami; in the fifth foot with the very small barely jointed spine on the second segment; but also in the spine formula for the four swimming feet which is 2, 3, 3, 3; and, most significant of all, in the shape of the seminal receptacle, the figure shown in the table for the European *viridis* being an exact picture of the same organ in the form under discussion.

And, lastly, the somatic chromosome number I have found to be 12, the same as that of the European *viridis*.

I see no reason why this form should not be entitled to the exclusive rights of the name *Cyclops viridis* Jurine; and *C. Americanus*, *C. parcus*, and *C. brevispinosus*, each with its distinctive chromosome number, spinal armature for the swimming feet, and seminal receptacle, be raised to the rank of separate species.

That the individuals I have just secured are not to be classed with *C. ingens* Herrick I conclude from Herrick's statement ('95) that the latter is merely an exaggerated form of *C. Americanus*. Neither are they to be compared with the forms which Miss Byrnes describes as *C. ingens* (?) for this latter species Miss Byrnes distinctly states as possessing the *brevispinosus* spinal armature of the swimming feet. My individuals, on the other hand, possess the *parcus* spinal armature for both the outer and inner rami of the swimming feet, and this armature is identical with that of the European *viridis*.

LITERATURE CITED

Braun, Alex.

'09 Die specifische Chromosomenzahlen der einheimischen Arten der Gattung *Cyclops*. Arch. f. Zellf., 3.

Byrnes, E. F.

'09 The Freshwater *Cyclops* of Long Island. Cold Spring Harbor Monographs, VII. Pub. by Brooklyn Inst. of Arts and Sc.

Claus, C.

'93 Neue Beobachtungen über die Organisation und Entwicklung von *Cyclops*. Arb. Zool. Inst. d. Univ. Wien, Bd. 10.

Gates, R. R.

'09 The Stature and Chromosomes of *Œnothera gigas* DeVries. Arch. f. Zellf., 3.

Haecker, V.

'97 Die Keimbahn von *Cyclops*. Arch. f. mikr. Anat., 49.

'03 Uber das Schicksal der elterlichen u. grosselterlichen Kernanteile. Jen. Zeitschr., 37.

Herrick, C. L., and **Turner, C. H.**

'95 Synopsis of the Entomostraca of Minnesota. Geol. Nat. Hist. Surv. of Minn.

Marsh, C. Dwight.

'10 A Revision of the North American Species of *Cyclops*. Trans. Wis. Acad. Sc. and Letters.

Schmeil, O.

'92 Deutschlands freilebende Süsswasser-Copepoden. I. Theil, Cyclopidæ. Bibl. Zool. (Leuckart u. Chun), Heft 11.

Wolf, Eugen.

'05 Die Fortpflanzungsverhältnisse unserer einheimischen Copepoden. Zool. Jahrb. Syst., 22.

NOTES ON THE HISTORY OF BARRED BREEDS OF POULTRY.[1]

RAYMOND PEARL.

At the present time modern breeds of poultry are being much used as material for the study of various problems in genetics. On this account matters connected with the early history of these breeds take on a general biological interest which they would not otherwise possess. It is the purpose of these notes to call attention to certain historical matters which bear directly upon some problems now occupying the writer's attention.

I. The Production of the Barred Color Pattern from the Mating of Solid Black with Solid White Birds.

In an earlier paper[2] from this laboratory the following statement was made respecting the origin of the barred color pattern, seen now in Barred Plymouth Rock and several other kinds of poultry.

"In regard to the origin of this barred color pattern in poultry very little can be learned. It is known that the Barred Plymouth Rocks owe their barring to the Dominique, which was used in the crossing which led to the production of this breed. But as to the source of the barring in the Dominiques we have found no evidence. Wright (1) says (p. 301) that: 'The colour (barring) itself is not a primary one, but the produce of white with either black or a very dark color. Such colours, mated together, produce as the result, on a wide average of cases, more or less of blacks, whites, mottles or splashes with the plumage of Houdans and Anconas, blues or blue duns like that of blue Langshans and Andalusians, and that bluish barred plumage known as Dominique in America and Cuckoo in England. When once produced, this last colour has however a strong tendency to permanence; and in the common native Dominique fowl of the

[1] Papers from the Biological Laboratory of the Maine Experiment Station, No. 36.

[2] Pearl, R., and Surface, F. M. "On the Inheritance of the Barred Color Pattern in Poultry." *Arch. für Entwicklungsmech.*, Bd. XXX. (Teil I.), pp. 45–61, 1910.

West Indies and United States it had been preserved and bred so long as to be of a very fixed type indeed, though even in these fowls there was a constant tendency for white or black feathers of the original components to appear, as well as the straw or red which always troubles breeders of white or black fowls.'

"This is the statement of a fancier, made without special study of the inheritance of barring. It is certainly correct in the statement that the barring has become firmly fixed in the Plymouth Rock at least. Such a thing as a completely non-barred bird appearing in any 'pure bred' strain of Barred Plymouth Rocks no longer occurs and has not for a number of years. The statement that the barred pattern originated from a cross between black and white birds, as a sort of intermediate condition, is, we feel certain, a mistake. The barring is a perfectly definite pattern, not simply a mixture of black and white, or a 'splashed' coloration such as is seen in Houdans. The inheritance of barring is of such character as to indicate most strongly that we have to deal here with a unit character, viz., a particular definite and characteristic pattern. Further, so far as we are aware, none of the experiments regarding the inheritance of color in poultry carried out by Bateson, Punnett, Hurst, Davenport[1] or the present writers give the slightest evidence that breeding black and white birds together will produce barred offspring. Finally, in the case of the Plymouth Rocks, where this pattern reaches its most perfect expression, the known history of the breed makes it certain that the barring was not created *de novo*, but was taken from the Dominique."

Since this was written I have found in the literature an interesting piece of definite circumstantial evidence regarding the appearance of the barred pattern in the offspring of a solid black and solid white bird mated together. This would seem at first glance to be clear proof for the *de novo* origin of the pattern. As such it is worth discussing. The case in point concerns the

[1] It should have been stated that Davenport (Carnegie Institution, Publication 52, p. 40) has reported barred offspring from crossing a White Leghorn bantam ♂ with a Black Cochin bantam ♀. His results from pure matings, however, show plainly, as he himself states (*loc. cit.*, p. 40 and p. 75), that the White Leghorn stock used carried the barred pattern factor. This case then evidently has no critical bearing on the point under discussion here.

origin of race of bantam fowls known among English fanciers as Cuckoo Pekins. The Cuckoo Pekins were originated about 25 years ago by the well-known English fancier and authority on the bantam breeds, Mr. William Flamank Entwisle. In his book[1] on bantams, which is the standard work on the subject in English, he gives the following statement regarding the appearance of the Cuckoo variety (*loc. cit.*, p. 40).

"We now pass on to the Cuckoo Pekins. These are a very recent introduction; in fact, the first time that a pair of this variety was exhibited was at the Bawtry Show in September, 1888, when Master Frank E. Entwisle exhibited three pairs of them, which produced quite a sensation, one pair winning the silver cup. In colour they are quite as perfect as any shown since, though they are now much improved in shape, cushion, softness of tail, and abundance of foot and shank feather. We first produced Cuckoos in this way: While crossing Black Pekins and White Booted with the double intention of strengthening the Blacks, and producing White Pekins, we reared, amongst others, one a rather dirty looking white, so very excellent in shape, etc., that we thought it good enough to show as White Pekin at the Dairy Show; so we had it caught and washed, but to our surprise it would not come a better white than when first put into the soapsuds; we tried a thorough good soaking, washing, and rinsing, and then had her carefully dried; and on the following morning we had a careful look at her, when we discovered faint but regular bars of stone colour, on a milk white ground. We at once saw that in this pullet we had a more valuable prize than a pure white would have been, and we mated her with her sire, a Black Pekin cock, for the next season. From this mating we had distinct cuckoo markings, and these pullets we mated with a Cuckoo cockerel, which Mr. Leno kindly sent us, and which he bred from his imported Chinese Cuckoo cock, we believe the only one ever sent from China. Then we bred in-and-in, and back to the pure Black Pekins, until they have proved themselves capable of, now and then, beating all other colours of Pekins."

In considering this case the first point to be noted is that there

[1] Entwisle, W. F., "Bantams." Wakefield, 1894 (?), pp. 1–116.

is every reason to suppose that entire reliance may be placed on the statements made, so far as they go. In other words, Mr. Entwisle may be regarded a reliable witness as to the facts. He held a distinguished place among British fanciers, and his book furnishes much evidence that he was a keen and careful observer. Of course, as is usual in such fanciers' reports, critical evidence is lacking at important points in the case here under discussion.

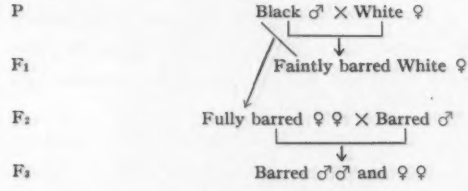
One thing, however, seems clear, namely that while in the particular instance cited, the barred pattern appeared in the F_1 generation from a mating of black by white, it is by no means certain that one or the other of the parents did not carry barring latent (*i. e.*, as a cryptomere). The difficulty in taking this case as proof of the *de novo* origin of the barring lies in the fact that a "Mr. Leno" had at that time a cuckoo cock "imported from China," which evidently carried the barred pattern in hereditary form. If this were the case it is obvious that other color varieties of Asiatic bantams might carry the barred pattern determiner or factor in their gametes, without its being somatically visible. That this is the true explanation of the case is indicated by the fact that in this pullet the *pattern* appeared at once in apparently almost perfect condition ("faint but *regular* bars"). This would scarcely be expected if what is occurring here is the beginning of the synthesis of a barred pattern from pure black and white. Rather one would suppose that at the outstart the barring would be irregular and indefinite in character.

This case described by Entwisle must, then, be regarded as failing to furnish *critical* evidence of the *de novo* origin of the barred pattern in fowls from crossing solid black and solid white.

Incomplete as are the data, however, the case is of interest in another direction. If it be assumed, for the reasons set forth above, that one of the parents of this faintly barred pullet carried the gametic determiner for barring, then one must conclude that it was the White Booted parent. The reasons are, (*a*) that in all cases now known at least (and they cover in published and unpublished work a fair number of different breeds of poultry), if

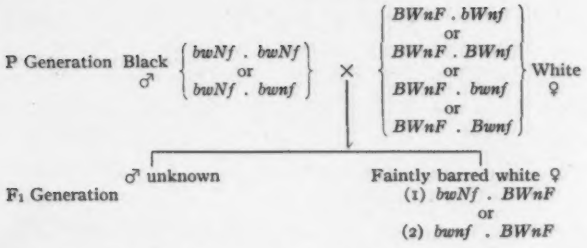
the factor[1] for black pigment and the factor for the barred pattern are present together the zygote will show the barred character. In other words there is no evidence that a black bird can carry barring latent. If such a bird has the barring factor at all it will be visible somatically, so far as present experience goes. (*b*) The white of the White Booted ♀ parent must have been "dominant white," from the account given. This is indicated by the fact that the prevailing color of the F_1 bird from the cross of this white ♀ with a black ♂ was white. A "dominant white" bird could carry barring gametically for an indefinite period without its showing somatically.

From the data given it is possible to construct the following pedigree.



Now in view of the fact that the barred pattern in all cases so far studied behaves as a sex-correlated character for which the female is heterozygous, this pedigree gives a remarkable result. If the barring was latent in the original White Booted ♀, which seems probable for the reasons set forth above, the observed results *can only be explained on the assumption that the factor for barring and the female sex determiner were carried in the same gamete.* Thus if *B* denotes presence of factor for barred pattern; *W* presence of factor for "dominant white"; *N* presence of factor for black; *F* presence of factor for ♀ sex, and the corresponding small letters the absence of these factors, the pedigree for this black × white cross would stand as follows.

[1] Or factors. Throughout the present discussion it will be assumed for the sake of verbal economy that the characters in each instance depend upon the action of a *single* gametic factor. It makes no difference to the argument whether this is or is not true in a particular instance.



Of these two alternative formulæ in F_1 the first is decidedly the more probable, since there is every reason to expect that (2) would be a pure white bird showing somatically no trace of barring.

In the F_2 generation got by mating the faintly barred white ♀ of F_1 to the black ♂ of the P generation the *females* were barred, indicating again that one of the gametes uniting to form these individuals must have borne both *F* and *B*, since no gamete from the sire could bear either of these factors.

It is evident that in interpreting this case we are forced to adopt either one or the other of two alternatives, both of which present novel points in comparison with the results of recent experiments regarding the inheritance of the barred pattern in crosses involving Barred Rocks, in which this pattern is well fixed. On the one hand we may conclude that the White Booted ♀ original parent carried the *B* factor in its gametes. This interpretation leads to the results worked out above, the novel point in which is that here there is no *repulsion* between *B* and *F* in gametogenesis (or *coupling* between *B* and *f* if one chooses that view) as is the case in Barred Plymouth Rocks of the present day. Here a non-barred ♂ mated with a female carrying barring (by hypothesis) produces *barred daughters*, where there should be produced (to accord with recent experiments on barring) barred sons and *non-barred* daughters. On the other hand it is possible to assume that the faint barring in the F_1 ♀ arose *de novo*, and that the White Booted ♀ parent did *not* carry the *B* factor. On this view it must be concluded that *this new character barring when it first appears behaves in an absolutely different way in inheritance from what it does later*. Either conclusion is sufficiently interesting, and stimulating to further research.

Of course a third assumption still is possible, namely that the barring of the Cuckoo Pekins is a different barring entirely from that of Barred Plymouth Rocks, and therefore behaves differently in inheritance. There is no evidence, however, on which to base such an assumption. All of the types of barring which do behave differently in inheritance from the Barred Rock type (*e. g.*, the Campine or the Pencilled Hamburgh barring) are somatically distinctly different from the Barred Rock type of

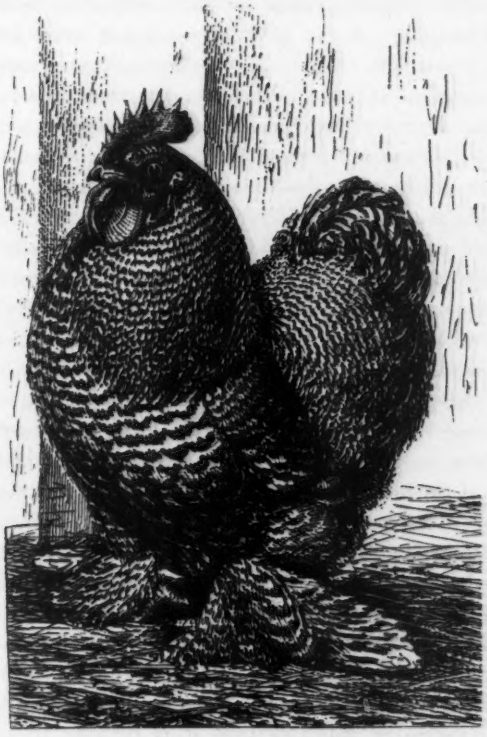


FIG. 1. A Cuckoo Pekin ♂, winner of first and special at Whitby, first and special at Hallam and Ecclesall, etc., etc., 1894. From Entwisle, *loc. cit.*, p. 20.

barring. The barring of the Cuckoo Pekin, however, belongs to the same type somatically. This is shown in Fig. 1, which is a copy of a picture of a Cuckoo Pekin ♂ drawn by the well-known

English poultry artist Ludlow, and published in a plate facing p. 20 of Entwisle's[1] book.

One of the most important and most baffling problems in genetics today is the analysis of "pattern" inheritance. There is definite and indubitable evidence that certain plumage patterns in poultry behave as clean-cut unit characters, dependent on gametic determiners or factors which act precisely like pigment factors for example. Yet the gametic and ontogenetic mechanism of pattern determination and development is most difficult even to imagine. There certainly is great need for further research in this field. Particularly it is important to see whether definite patterns may be formed *de novo* from crosses of birds which bear no trace of the determiners of the patterns gametically.

The case here under discussion illustrates the difficulties which attend the getting of really critical evidence on this matter for the barred color pattern. Merely to show that a black and white bird mated together give barred offspring will not suffice. This happens if one mates any bird carrying black pigment with a White Plymouth Rock, but it is merely (and obviously) because the White Rock carries the barred pattern factor as a cryptomere. To get crucial evidence one must use black and white breeds (*a*) in which there is no evidence of barred birds having been used in the crosses from which the breeds were originated; (*b*) in which there never occur barred "sports"; (*c*) in which barred varieties of the breed are unknown; and (*d*) in which the white of the white parent is a "recessive" and not a "dominant" white. These criteria at once exclude from experiments on synthesizing the barred pattern from black × white crosses, if such experiments are to be really critical, all Mediterranean breeds (so far at least as these breeds are known to the writer). Further the stock used must be given a thorough preliminary test in Barred Rock crosses to determine whether it does or does not carry the *B* factor. Experience indicates that it is difficult to settle this point if the white of the white race belongs to the "dominant white" category. There are, however, certain races of poultry which seem to fulfil the requirements for a crucial test of the fundamental question of the *de novo* formation of the barred

[1] *Loc. cit.*

pattern from a black × white cross. Experiments are now being carried on in this direction by the writer.

II. The Color and Pattern of Early Barred Plymouth Rocks.

The first Barred Plymouth Rock fowls to be entered under this name at a poultry show were exhibited in 1869 by D. A. Upham at Worcester, Mass.[1] They had first been bred some four or five years before that time. So far as I have been able to learn the earliest published picture of fowls of this new breed of fowls, which was in any degree an accurate or adequate representation of the actual birds, first appeared in the *American Agriculturist* in January, 1872.[2] The well-known poultry artist Mr. Franklane L. Sewell states[3] that he knows of no earlier picture than this and his experience in this field is extensive. Whether this picture is absolutely the first of the breed to appear is not essential, nor of any interest other than purely antiquarian. The biological interest of the picture lies first in the fact that it shows the appearance of birds of this breed very early in its history, and second in the fact that the drawing is well done, and may be taken with considerable confidence as an accurate representation of the appearance of the best of the Plymouth Rock fowls of that time. This wood-cut, which is here reproduced as Fig. 2, bore originally the initials, "E. F.," which were those of Edwin Forbes, a rather clever delineator of poultry, who worked in New York.

The picture was copied, or rather apparently printed from the same block, without credit for a prior appearance, and with the artist's initials erased, in the *Poultry World*[4] for July, 1872. It is from this publication that the picture has been chiefly known to poultry fanciers. Sewell[5] gives the *Poultry World* as the original place of publication.

[1] Robinson, J. H. "Principles and Practice of Poultry Culture." Boston, 1912, Ginn & Co., p. 399.

[2] Vol. 31.

[3] Sewell, F. L. "Color of Plumage of the Barred Plymouth Rock," *Reliable Poultry Journal*, Vol. XV., p. 520, July, 1908.

[4] Vol. I., p. 85. This journal which has long since ceased to exist, was published at Hartford, Conn.

[5] *Loc. cit.*

The drawing was made from actual birds, and is therefore not wholly imaginary or idealistic. The pair of birds figured belonged to a Mr. C. C. Corbett, of Norwich, Conn., and represented a high degree of excellence for the time.

The chief points of interest in this picture for the student of genetics are the relatively dark color of the birds and the indistinctness of the pattern. In both respects, of course, these birds

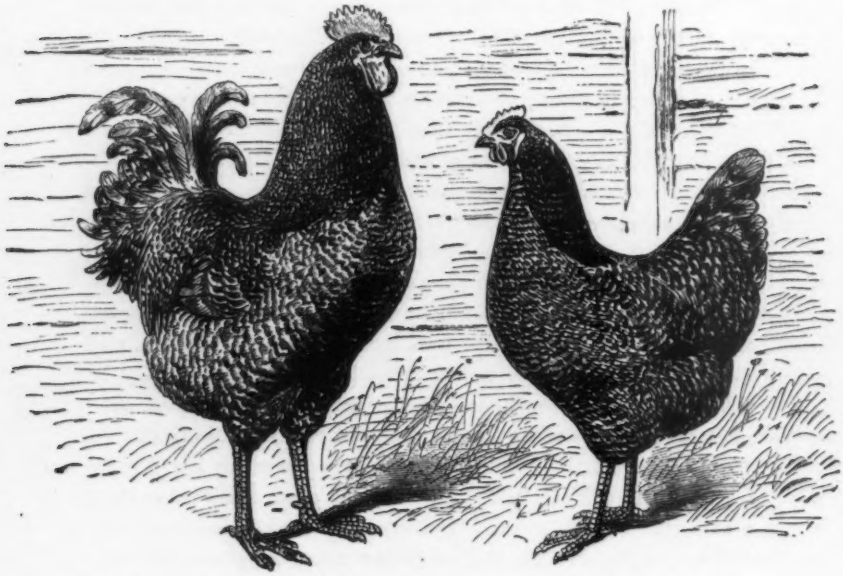


FIG. 2. Plymouth Rock fowls as they appeared in 1872. This is believed to be the earliest adequate picture of birds of this breed.

stand in marked contrast to the Barred Rocks of the present day. While a generally barred effect is evident, the pigment obviously spreads over a great deal of the feather, encroaching on the areas which are white in a modern Barred Rock.

The fact to which I wish especially to call attention is that *these early Plymouth Rocks were evidently very similar indeed in color and color pattern to the F_1 birds obtained by crossing a modern Barred Rock ♂ with a ♀ belonging to some heavily pigmented breed, such as for example the Cornish Indian Game.* Allowing

for differences between modern photographic technique and a wood-cut printed on poor paper, and for differences in shape of body, due to the game blood, the similarity between the birds in Fig. 2 and the barred ♂ and ♀ of the F_1 generation of the cross B. P. R. ♂ × C. I. G. ♀ published in Roux's Archiv[1] is striking. The male plumage is relatively dark in both cases as compared with that of the modern pure B. P. R. ♂. In the female there is also an excess of pigment, seen not only in the general color tone

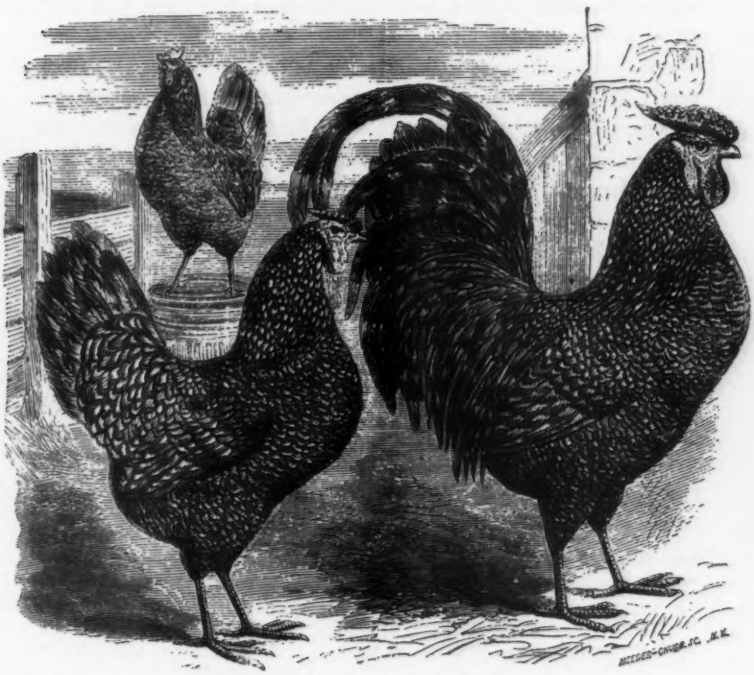


FIG. 3. Reproduction of a wood-cut of Dominiques, originally published in 1870.

of the bird, but in the apparent "smokiness" due to the encroachment of the black bars on to the white areas of the individual feather.

Now in the case of the F_1 birds it is clear what causes (or is at least associated with) the defective development or expression

[1] Pearl and Surface, *loc. cit.*

of the pattern. The pure B. P. R. ♂ of today is, so far as is known, always homozygous with reference to the barring factor, whatever that may be. He carries two "doses" of *B*. Somatically he is light in color with narrow clean cut bars. When by crossing a male is made carrying but one "dose" of *B* (*i. e.*, heterozygous in relation to the barring factor) the somatic pigmentation is markedly changed, and becomes like that of the earliest Barred Rocks known. This obviously suggests that in the early history of the breed the males were regularly heterozygous with reference to barring. If so they should have produced, with considerable regularity, non-barred (black) daughters. As a matter of fact this was probably the case. Up until 20 years ago, and even later in some localities, one would judge from various statements to be found in poultry journals, agricultural papers and the like, that it was not a particularly uncommon occurrence for a Barred Rock mating to throw some solid black chickens.

It is of interest to note that at the time of the original foundation of the Plymouth Rock breed the Dominiques, from which the barred pattern was derived, had the same type of pigmentation. This is shown in a contemporary wood-cut of the latter breed, reproduced in Fig. 3.

This picture of Dominique's was published in the *American Agriculturist*, Vol. 29, p. 13, 1870. It was drawn by Edwin Forbes from a pair of birds owned by Col. Henry Howland of Chicago. These birds were prize winners in their time. This cut, in a very much garbled form, was reproduced in the *Fanciers Journal* in 1876, from which source it has been copied by Sewell.[1]

[1] *Loc. cit.*

COMPLETE DISCHARGE OF MITOCHONDRIA FROM THE SPERMATOZOÖN OF PERIPATUS.[1]

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The following account presents the unique case of a spermatozoön that loses all its mitochondria with the abstriction of the cytoplasm.

In my paper ('00) on the spermatogenesis of *Peripatus balfouri* the history of the germinal cycle was described from the spermatogonia through the maturation divisions, and that is now completed by a description of the spermiogenesis. In that account certain distinctive bodies were figured and discussed under the name of "yolk spherules." These were noted to occur only sparsely in the spermatogonia, but to become abundant during the growth period of the spermatocytes, and in all mitoses to lie outside of the spindles. Similar bodies were seen in the sheath cells of the testis, and I concluded these to be nurse cells, elaborators of the supposed yolk spherules, and that the spermatogonia received their "yolk spherules" from these nurse cells. At the time when that paper was sent to press Benda had not yet published his term mitochondria, and in conformity with the results of other investigators of that day I supposed true yolk to be formed within spermatocytes.

Now I am able to demonstrate that the bodies in question are not yolk spherules, rather chemically quite different from these, but are mitochondria according to their behavior and staining reactions. Indeed, it will probably be found that most bodies described as yolk spherules in spermatocytes are really mitochondria. My early account was therefore one of the first to describe mitochondria through successive cell generations of spermatogenesis. But that account was wrong in its inference that those of the germ cells proper are derived from those of the nurse cells; on the contrary, they occur independently in the

[1]*Note by the Editor.* Professor Montgomery died while this paper was in press. He had therefore no opportunity to make any changes in the proof, and the paper is printed exactly as the manuscript left his hands.

two classes of cells, just as they do in the Sertoli cells and spermatocytes of mammals.

The material consisted of testes, seminal vesicles, vasa deferentia, and oviducts, some fixed in strong Flemming's fluid diluted with an equal part of distilled water, and others preserved in corrosive sublimate-acetic; all were originally received from my friend, Dr. Purcell, of Cape Town. The mitochondria appear pale red after Hermann's safranine-gentian violet, and after the Ehrlich-Biondi-Heidenhain method; shades of gray or black after iron hæmatoxylin, according to degree of destaining; and after Benda's stain they are deep violet, while the chromatin is brownish and the centriole red—the typical reaction to this stain. There is only one other object known to me on which they are equally readily demonstrated, namely, the spermatocytes of *Ascaris*.

Fig. 1, Pl. I., exhibits the position of the mitochrondia at the end of the second maturation mitosis where, as after the first also, they lie at the distal poles (equatorial ends) of the daughter cells. Until their later fusion takes place they are chiefly peripheral, next the cell wall, spherical or slightly elongate, and in the form of hollow vesicles. In the earliest spermatids they always form a layer at the distal pole, but sooner or later move forward, along the cell membrane, so as to take a position on the side of the nucleus (Figs. 2–16); at the same time the sphere (*s*) always advances from its original position and the cytoplasm comes to make a lobe around the nucleus and entirely in front of the centriole (*c*). These movements do not occur synchronously on cells of the same stage, there is much variation in the process, yet the end result is the same in all. By reason of the mitochondria remaining generally in a single layer they may be readily counted, and their number is found to differ in different spermatids, which shows their mass cannot be accurately quartered by the maturation divisions. In Figs. 4, 6, 11–13, all of each cell are drawn with care, and their numbers here are respectively: 33, 45, 68, 64, 49. Their volumes also differ considerably, as the figures show. They do not blacken with osmic acid.

In the nucleus the chromosomes are at first peripheral and quite distinguishable (Fig. 2), then coalesce to produce a hollow

chromatin sphere enclosing clear karyolymph (Fig. 3). Next the nucleus lengthens, and distally its chromatin border becomes very thin (Figs. 4, 5, 7, 8); but it could not be determined that at this thin region nuclear sap passes out of the nucleus in the way I have described (1911) for *Euschistus*. This thin area of the nuclear wall later becomes as thick as the remainder (Figs. 9, 10). Then the proximal end of the nucleus becomes pointed (Fig. 10). With its later great elongation (Figs. 11–17, and Plate II.) the nucleus changes its appearance, due apparently to its interior becoming more chromatic, so that on surface views it appears nearly homogeneous throughout; it continues the same affinity for basic stains, and from the stage of Fig. 11 onwards I have drawn it brown and not deep black simply in order to represent the mitochondria more distinctly. Yet cross sections show that even in the mature sperm the chromatin makes a hollow cylinder and not a solid rod.

Now to return to the mitochondria, to describe their particularly notable phenomenon. After all of them have moved forward from the distal pole, carried probably by currents in the lobe of cytoplasm, they fuse together to produce a true Nebenkern or chondriosome (Meves, '00). Figs. 11–16 show them becoming agglomerated, and in Fig. 17 they are seen to be fusing to produce compound vesicles. Fig. 18, Pl. II., exhibits them on the side of the nucleus, with fusion far advanced, and Fig. 19, their consolidation into a chondriosome. As the process of fusion advances they stain more deeply, so that the chondriosomes when completed appear densely chromatic (Figs. 20–22). Sometimes a few mitochondria remain isolated without joining with the others, sometimes all fuse together (Figs. 18–21). Simultaneously the cytoplasmic lobe moves forward along the nucleus, or, probably more correctly, the nucleus moves backward through it; and in its substance appear denser strands and minute granules (Figs. 18, 19) which may be degeneration products comparable with the "tingible granules" of mammals. Consequently, each nearly mature sperm of the seminal vesicle (Figs. 20, 21), as all of the vas deferens (Fig. 22), carries near the anterior end of the nucleus a cytoplasmic lobe with a densely staining chondriosome; there appears to be no cytoplasm at all

in the region of the centriole and the flagellum. Were the history of the spermatozoa unknown beyond their conditions in the vasa deferentia, there would be no evidence of the fate of the cytoplasmic lobes and chondriosomes. But fortunately I have numerous preparations of oviducts from female individuals, all crowded with spermatozoa, and in these all the spermatozoa lack entirely the cytoplasmic lobes and chondriosomes (Figs. 23, 24); in not a single case was a cytoplasmic lobe observed upon a spermatozoön when within an oviduct.

Peripatus, accordingly, has for us more than a phylogenetic interest, it has a high cytological importance. The spermatozoön during its development casts off its cytoplasm, and evidently all of it. But this abstriction of the cytoplasm, or a portion of it, is now known to be a quite general phenomenon in animals, and only amphibians and certain insects appear to furnish exceptions to it. Much more important is that all the mitochondrial substance, in the form of a compact chondriosome, is cast away with it. Further, I had previously described the spermatozoön as possessing a lance or perforatorium, staining differently from the nucleus. Now I can demonstrate that this supposed perforatorium stains differently only on account of its excessive tenuity, that it is only the narrowed proximal end of the nucleus, and that it has no connection with the sphere. We have seen that the sphere arises just behind the nucleus (Figs. 2, 3), and moves forward into the cytoplasmic lobe (Figs. 5, 7, 8, 10, 12, 14, 16–19). When the chondriosome is fully developed the sphere lies still in the cytoplasmic lobe, separated from the nucleus (Fig. 20), and no evidence was observed that it moves along the latter to constitute a perforatorium. Therefore it is certain that the sphere as well as the chondriosome becomes thrown off with the cytoplasm.

The history of the centriole was not followed in detail. In the telophase of the secondary spermatocytes a minute centriole is present at each pole (Fig. 1). At the next stage when it was noticed (Fig. 3) it appeared as a much more voluminous body at the distal pole of the nucleus, and it retains this position thereafter. Later it becomes discoidal with indication of subdivision into two parts (Figs. 4–10), and afterwards lengthened in the axis

of the spermatid (Figs. 11–19), reaching its maximum size at the stage of Figs. 13, 14. In the mature and nearly mature spermatozoa it makes a slender rod, joining nucleus and flagellum, and then is seen to have decreased in volume (Figs. 21–24). The flagellum connected with it is a delicate, flattened thread, evidently without spiral membrane or cytoplasmic sheath; in the figures only its proximal portion is shown. In some cases there appeared to be a spiral skeleton around the nucleus, such as Koltzoff (1908) has recently described in other species; but examination proved that in *Peripatus* this is occasioned simply by chance wrapping of a flagellum around the nucleus.

In the mature egg and in cleavage stages no structures were found in any way resembling the mitochondria of the sperm cells.

Meves (1908, and later papers) draws the conclusion that mitochondria are important hereditable elements, directing cytoplasmic activities as the chromosomes direct those of the nucleus, self-perpetuating bodies differentiating during ontogeny into most of the fibrillar structures of the body. Without entering into the rapidly growing literature now, we will be content with the statement that a considerable number of investigators corroborate these views, and that they have been especially elaborated by Giglio-Tos and Granata ('08). This hypothesis more than any other has directed attention to these bodies. Yet they are far less conservative and regular than the chromosomes in number, form and behavior and there is evidence that occasionally some of them are eliminated during spermiogenesis. Thus Fauré-Fremiet has distinguished four types of them: (1) Those that undergo changes of position without profound morphological changes, as in mammalian spermiogenesis. (2) Those that at the same time undergo great structural changes, as in insect spermatids. (3) Those of which only a part change into the chondriosome or Nebenkern of the spermatid, while others degenerate, as in spermatogenesis of certain gastropods. And (4) those that transform wholly or partly into deutoplasmic bodies, some cases of oögenesis. The fourth of these classes cannot be said to be definitely established, but there can be little doubt about the evidence for the third. Thus, besides the instance in gastropod spermatogenesis studied by Fauré-

Fremiet, Retzius noted in mammals a "considerable reduction in their substance as they enter into the formation of the spiral thread." Jordan ('11) found that in spermatids of the opossum a considerable number of mitochondria are cast off with the cytoplasm; and while Duesberg ('10) maintains that in the guinea pig all take part in forming the spiral thread of the spermatozoön, yet he figures granules with similar staining reactions in the dehiscent cytoplasmic lobe. The parallel does not seem yet to have been made, yet may not the "tingible corpuscles" of mammalian spermatids be metamorphosed mitochondria, ones that have nothing to do with the spiral threads? And now we are able to adduce the positive case of *Peripatus*, in which all the mitochondria become removed from the spermatozoön.

In view of these facts it seems to me we should be very cautious in attributing to the mitochondria a rôle in cellular activity at all equal to that of the chromosomes. No spermatozoön ever discards chromosomes, but that of *Peripatus* throws off all its mitochondria.

LITERATURE CITED.

Duesberg.

'10 Nouvelles recherches sur l'appareil mitochondrial des cellules séminales. Arch. Zellf., 6.

Fauré-Fremiet.

'10 Étude sur les mitochondries des Protozoaires et des cellules sexuelles Arch. Anat. Micr., 11.

Giglio-Tos e Granata.

'08 J mitocondri nelle cellule seminali maschili di *Pamphagus marmoratus* (Burm.). Biologica, 2.

Jordan.

'11 The Spermatogenesis of the Opossum, etc. Arch. Zellf., 7.

Meves.

'00 Ueber den von v. la Valette St. George entdeckten Nebenkern (Mitochondrienkörper) der Samenzellen. Arch. mikr. Anat., 56.

'08 Die Chondriosomen als Träger erblicher Anlagen. Ibid., 72.

Montgomery.

'00 The Spermatogenesis of *Peripatus balfouri* up to the Formation of the Spermatid. Zool. Jahrb., 14.

Montgomery.

'11 The Spermatogenesis of an Hemipteron, *Euschistus*. Journ. Morph. 22.

Explanation of Plates.

All figures were drawn with the cameral lucida at the level of the base of the microscope, Figs. 13, 20 with Zeiss apochr. 1.5 mm., oc. XII., the others with Zeiss achr. 1/12 mm., oc. XII. Figs. 1–3, 5, 7–12, 14–19 from a seminal vesicle, Flemming's fluid, iron hæmatoxylin; Figs. 4, 6, 21 from a seminal vesicle, corrosive sublimate-acetic, iron hæmatoxylin; Figs. 13, 20 from a seminal vesicle, Flemming's fluid, Benda's stain; Fig. 22, from a vas deferens, Flemming's fluid, safranine-gentian violet; Fig. 23, from an oviduct, treated like the last; Fig. 24, from an oviduct, corrosive sublimate-acetic, iron hæmatoxylin.

c, centriole.

s, sphere.

Plate I.

Fig. 1. Telophase of second maturation mitosis.

Figs. 2–17. Successive stages of spermiogenesis from the seminal vesicle. Fig. 6 is an apical view of the stage of Fig. 5.

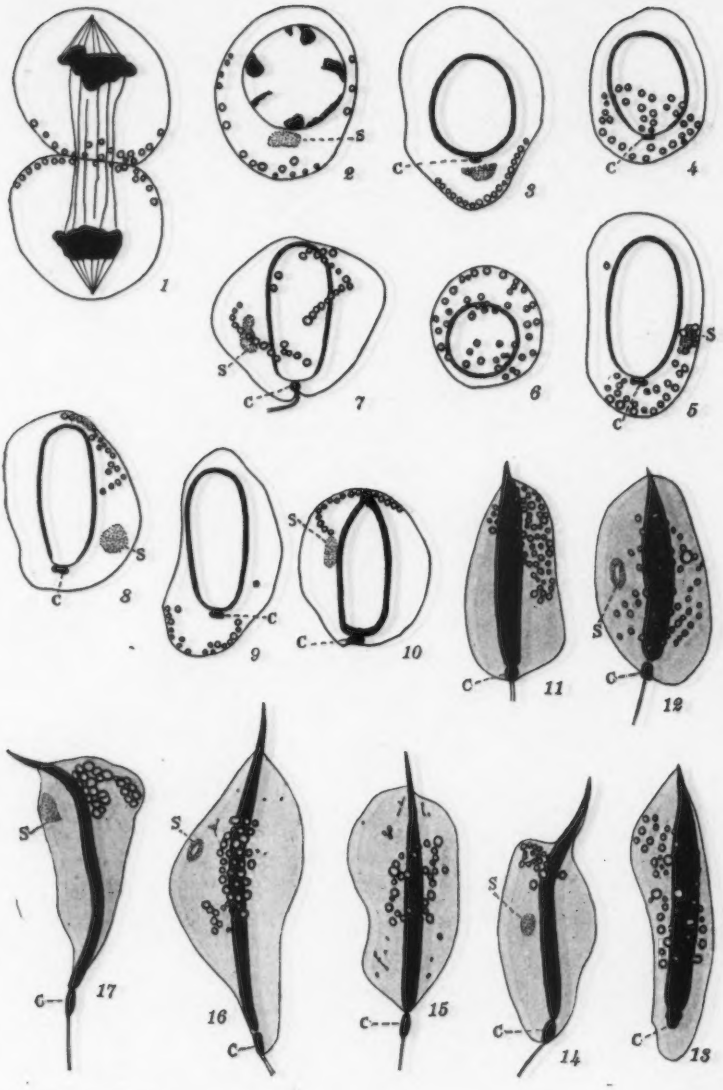

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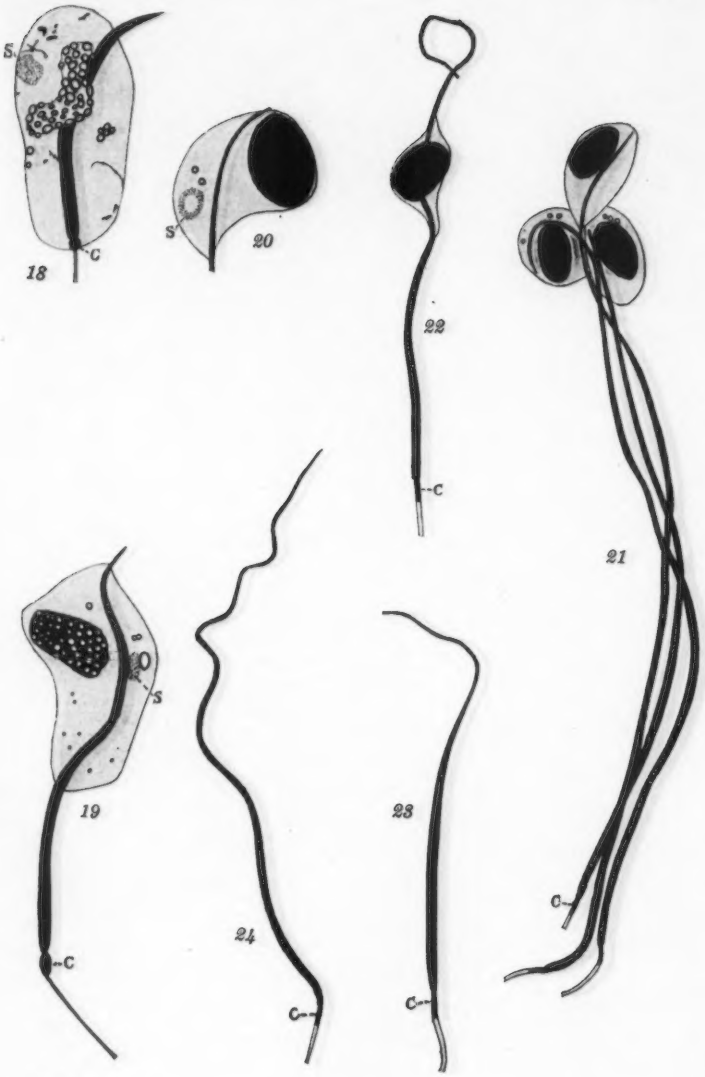
PLATE II.

FIGS. 18–21. Later stages of spermiogenesis from seminal vesicle. In Fig. 20 only the cytoplasmic lobe and the proximal portion of the head are shown.

FIG. 22. Spermatozoön from vas deferens.

FIGS. 23, 24. Spermatozoa from oviduct.



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